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Inhibition of Marrow CFU-E Colony Formation From Human Immunodeficiency Virus-Infected Patients by β- and γ-Interferon

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Increased production of cytokines such as β-interferon (IFN) and γ-IFN may contribute to the anemia frequently observed in patients with human immunodeficiency virus (HIV) infection. The hypothesis that HIV infection might enhance the susceptibility of erythroid progenitors to cytokine-mediated inhibition was evaluated by comparing the effects of β- and γ-IFN on in vitro colony formation by marrow erythroid colony-forming units (CFU-E) from HIV patients, normal volunteers, and anemic non-HIV-infected individuals. CFU-E colony formation from HIV patients was not significantly different from controls, and the degree of inhibition by IFN did not differ among patient subsets. HIV infection does not appear to impair baseline CFU-E colony formation, nor does it appear to enhance the susceptibility of CFU-E to suppression by cytokines. © 1996 Wiley-Liss, Inc.

Key words: anemia, cytokines, erythropoiesis, human immunodeficiency virus, interferon

INTRODUCTION

Anemia is common in patients with human immunodeficiency virus (HIV) infection [1]. It has been suggested that increased production of cytokine mediators of the immune or inflammatory response, such as tumor necrosis factor (TNF) or interleukin-1 (IL-1), may contribute to the development of this anemia [2,3]. It has been demonstrated previously that TNF and IL-1 exert their inhibitory effects on colony formation by human erythroid colony-forming units (CFU-E) indirectly, via β-interferon (IFN) and γ-IFN, respectively [4,5]. The hypothesis for this investigation is that HIV infection of erythroid progenitors may cause them to be more susceptible to the inhibitory effects of β- and γ-IFNs. In order to evalute this possibility, the effects of β- and γ-IFN on CFU-E colony formation by marrow cells from HIV patients were determined.

MATERIALS AND METHODS

Marrow-Cell Collection and Processing

This study was approved by the University of Cincinnati Medical Center Institutional Review Board. Bone marrow (2–5 ml) was aspirated from the posterior iliac crest of patients undergoing diagnostic bone-marrow examination or from healthy paid volunteers, and collected in 5 ml of Iscove's modified Dulbecco's medium (IMDM; Sigma Chemical Co., St. Louis, MO) containing 10 U sodium heparin/ml. Marrow cells were then resuspended in IMDM and enriched for light-density mononuclear (LDMN) cells by separation over 1.077 g/ml Histopaque (Sigma).

Culture of CFU-E in Plasma Clots

Marrow LDMN cells were cultured at concentrations of 1×10^5 cells per ml with IMDM, 25% fetal calf serum (Hyclone Laboratories, Logan, UT), 1% bovine serum albumin (Sigma), rhEPO 1 U/ml (Ortho Biotech, Raritan, NJ), penicillin, gentamicin, 1.5 mM epsilon aminocaproic acid, 1.3 mg/ml fibrinogen (Chromogenix AB, Chromogenix, Mölendal, Sweden), 0.2 U/ml thrombin (Parke-Davis Pharmaceuticals, Morris Plains, NJ), and various concentrations of recombinant human (rh) β-IFN (Berlex Laboratories, Richmond, CA) or rhγ-IFN (Genzyme, San

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Francisco, CA). Cells were cultured for 7 days at 37°C in 5% CO_2/95% air and then fixed and stained with benzidine-hematoxylin, as described by McLeod et al. [6]. CFU-E were defined as giving rise to colonies of 8–49 hemoglobinized cells [7]. All cultures were performed in duplicate, representing 6–8 plasma clots per experiment. Results are expressed as percentage of control (colonies cultured without IFN), to allow different experiments to be compared.

RESULTS

The 5 HIV-infected patients had significant hematologic abnormalities, with median hemoglobin 8.1 g/dl (range, 7.1–15.0 g/dl), and median platelet count 91,000/μl (range, 16,000–471,000/μl). The one HIV-infected patient who was not anemic had a platelet count of 33,000/μl. Median CD4 lymphocyte count was 124/mm^3 (range, 0–344/mm^3). Two patients had non-Hodgkin's lymphoma, 1 patient had Hodgkin's disease, and 2 had disseminated mycobacterial infection diagnosed at time of marrow examination. Three HIV-infected patients were receiving antiretroviral therapy at time of marrow examination (2 were receiving dideoxycytosine, and 1 was receiving azidothymidine). Effects of rhβ-IFN and rhγ-IFN on CFU-E colony formation by marrow from 5 HIV-infected patients and 4 normal volunteer donors (controls) are shown in Figure 1. Two patients with sickle-cell anemia and a patient with alcohol-related anemia served as non-HIV-infected anemic controls. No significant differences in degree of inhibition for a given concentration of either β- or γ-IFN were observed between any groups. In addition, colony formation in the absence of interferon did not differ significantly between HIV patients and normal controls ($P = 0.08$; Mann-Whitney test).

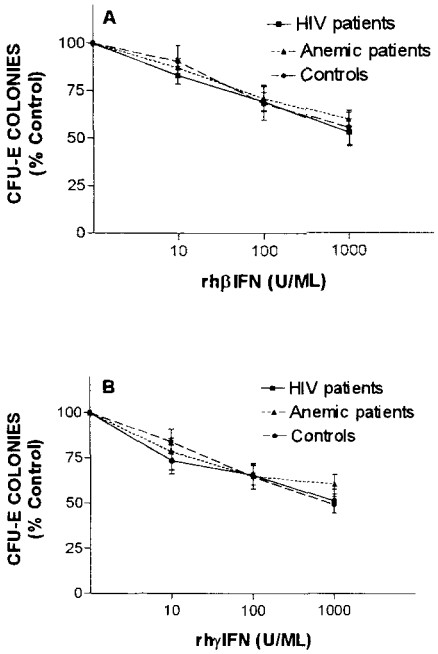

Fig. 1. Effects of rhβ-IFN (A) and rhγ-IFN (B) on marrow CFU-E colony formation by HIV-infected patients (n = 5), healthy normal volunteers (controls) (n = 4), and non-HIV-infected anemic patients (n = 3). Results are mean ± SEM. CFU-E colony formation in the absence of IFN was 694 ± 144 colonies/10^5 LDMN marrow cells, and 313 ± 99 colonies/10^5 LDMN marrow cells for HIV-infected patients and for healthy normal controls, respectively ($P = 0.08$).

DISCUSSION

Results indicate that CFU-E from patients with HIV and hematologic abnormalities are not more sensitive to cytokine-mediated inhibition of colony formation than are CFU-E from normal individuals or from non-HIV-infected anemic patients. In addition, CFU-E colony formation appears to be well-preserved in these patients with evidence of advanced HIV disease (opportunistic infection, lymphoid malignancy, anemia, or thrombocytopenia). This extends the observation by Neal et al. [8] that CFU-E colony formation by asymptomatic patients with early HIV infection was not impaired, and that the hematopoietic progenitor pool was not a major reservoir for HIV.

Studies of the effects of cytokines such as TNF, IL-1, and IFN on erythropoiesis in the anemia of chronic disorders have led to the development of a cytokine-mediated anemia model for this syndrome [9]. The studies described here support the applicability of this model to the anemia observed in HIV infection. In this model, HIV infection would contribute to anemia by enhancing cytokine production, rather than by direct effects on erythroid progenitors.

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